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Seasonal Changes in Ovarian Response to Photoperiods in Orange-Red Type Medaka[1]

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ABSTRACT—Three groups of orange-red type medaka, *Oryzias latipes,* were reared from October under different temperature-photoperiod regimes: natural condition, 18°C natural daylength and natural temperature 15L (15 hr of light/day). Pairs from these groups were periodically transferred to 18°C 15L or to 18°C 10L and kept separately. In natural condition group, ovaries of the fish transferred in mid- or late October kept immature even after one month under both regimes. In the fish transferred in late November or late December, ovarian growth and spawning were observed in about one month only under 18°C 15L. The fish transferred in early February matured in three weeks and spawned irrespective of photoperiod. Thus, ovarian refractoriness in autumn changed into long-daylength dependent maturation in winter, which was followed by photoperiod independent maturation in spring. In the fish transferred from 18°C natural daylength and natural temperature 15L in late December and early February, rapid ovarian growth occurred under 18°C 15L, but not under 18°C 10L. These results demonstrate that ovarian response to temperature-photoperiod conditions can be markedly influenced by environmental careers in medaka.

INTRODUCTION

Annual reproductive cycles of temperate-zone teleosts have been believed to depend upon seasonal changes in environmental factors such as photoperiod and water temperature. Effects of the environmental factors on the gonads have been studied in many fishes [1–5], and seasonal changes in gonadal responses have also been reported in cyprinids, gobiids and the stickleback [2, 6, 7].

Medaka, *Oryzias latipes,* is a small freshwater fish native to Japan and adjacent areas, and its spawning season extends from mid-April to early September [8–10]. Our previous study showed that only temperature rise in spring was necessary for the spawning season of medaka to begin and photoperiods had little effects on the gonadal growth [11]. The experiments started in late February, when yolk vesicle formation of ovaries had almost finished. In other seasons, however, effects of temperature-photoperiod conditions on gonadal growth might be different from those in spring.

The present study was conducted to examine seasonal changes in effects of temperature-photoperiod regimes on gonadal growth of medaka, mainly for female. The fish were reared under natural condition or conditions with a warm temperature or with a long daylength. Part of them were transferred to long (18°C 15L) or short daylength (18°C 10L) condition almost monthly from October to February and gonadal responses were examined.

MATERIALS AND METHODS

Orange-red type medaka, *Oryzias latipes,* born in the preceding breeding season were obtained in Tokyo in July. They were kept in a circulating outdoor tank (90×180×50 cm) in University of Tokyo till experiments started and fed on tubifex (freshwater oligochaetes) to satiation. All experiments were carried out in Tokyo.

In October, the fish of 26 to 30 mm in standard

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length were divided into the following three experimental groups, i.e. natural condition, 18°C natural daylength and natural temperature 15L (15 hr of light/day). Natural condition group consisted of 200 fish of each sex, which were kept in another circulating outdoor tank from October 17 under natural temperature and daylength. For the 18°C natural daylength group, 110 fish of each sex were evenly distributed to 11 circulating glass-aquaria (30×45×30 cm) regulated at 18°C under natural daylength on October 29, when natural water temperature was around 18°C. For the natural temperature 15L group, 110 fish of each sex were transferred on October 17 to two light-proof circulating tanks (80×170×50 cm) given a photoperiod of 15L by two 20 W fluorescent lamps and no temperature regulation. The fluorescent lamps were placed 30 cm above the water surface. All experimental tanks and aquaria were provided with water plants, *Ceratophyllum demersum.*

Eight fish of each sex were monthly collected at random from each group to examine changes in gonadosomatic index (gonad weight×100/body weight: GSI). The fish were anesthetized with MS 222 and the body weights were measured. After decapitation, the gonads were taken out to measure their weights. Ovaries of three females from each group were dissociated in carp saline [12], and diameters of largest 50 oocytes were measured using a micrometer. Spawning was checked every week in 18°C and 15L groups by replacing water plants with artificial spawning beds for three days in aquaria or tanks after cleaning. If spawned during the three days, all eggs attached to the spawning beds or dropped on the bottom were counted.

From these three groups, 15 to 4 pairs were periodically transferred to 18°C 15L or to 18°C 10L and kept separately. In the natural condition group, this transfer was conducted monthly from mid-October to early February to a circulating glass-aquarium (30×45×30 cm) on each regime. The aquaria of each regime were placed in a light proof bath (90×180×50 cm) provided with two 20 W fluorescent lamps placed 15 cm above the water surface. In the 18°C natural daylength and natural temperature 15L groups, the transfer to the aquaria of the same type was conducted in late December and early February. About half of the fish was randomly collected one month after the transfer. The rest was reared till mid-March and sacrificed. Changes in GSI and oocyte diameters were examined and spawning was checked in the same way as mentioned above.

RESULTS

Natural condition group

Under natural condition, average GSI of female gradually increased from 2% in October to 4% in March (Fig. 1), accompanied by slight increment of oocyte diameters (Fig. 2). In April female GSIs rose rapidly, and spawning began.

The transfers of the fish from natural condition

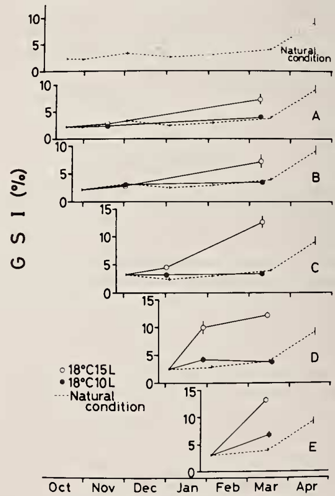


Fig. 1. Changes in gonadosomatic index (GSI) of female *Oryzias latipes* reared under natural condition, and transferred to 18°C 15L (15 hr of light/day) or to 18°C 10L. The transfer was conducted almost monthly from October to February. Solid lines in graphs A, B, C, D, E, indicate changes in GSIs of female transferred to 18°C 15L or to 18°C 10L. Dotted line in every graph shows GSI of female reared under natural condition.

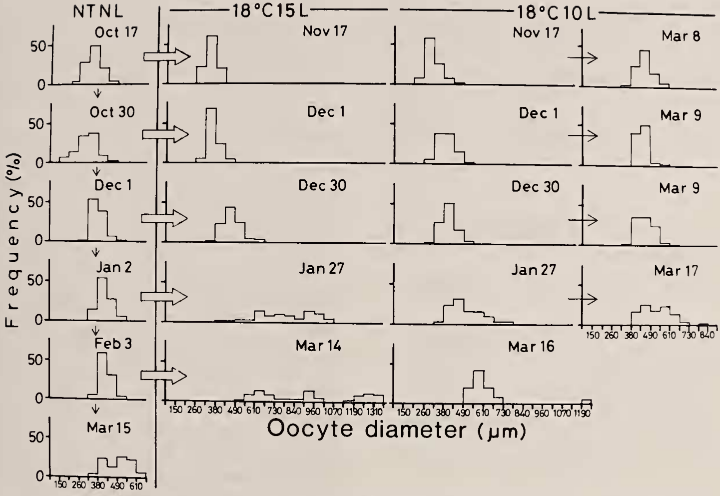


FIG. 2. Changes in distribution of oocyte diameters under natural condition (NTNL) and after the transfer to 18°C 15L or to 18°C 10L. The transfer was conducted almost monthly, and distributions of oocyte diameters under 18°C 15L or 18°C 10L were examined in one month and at the end (18°C 10L). Oocyte diameters under natural condition were examined within two days after the transfer.

to 18°C 15L or to 18°C 10L caused different responses in ovarian activity with time of transfer. When transferred in mid- or late October, ovarian growth was hardly observed during one month under both 18°C 15L and 18°C 10L. Under the long daylength, however, spawning started in January (Fig. 3) and GSI reached 7% in March (Fig. 1A, B). Under the short daylength, females did not mature until the end of the experiment with only slight increase in oocyte diameters (Fig. 2). Transfer in late November caused a little increase of GSI and oocyte growth under 18°C 15L in one month (Figs. 1C, 2), and resulted in active spawning from January (Fig. 3) and GSI of 12 to 13% in March (Fig. 1C). Under 18°C 10L, ovaries grew little until the end of the experiment (Figs. 1C, 2). Average GSI of the females transferred to 18°C 15L in late December increased to around 10% in one month (Fig. 1D), having oocytes large enough for ovulation (Fig. 2). Spawning began in early February (Fig. 3). Under 18°C 10L, females showed some increase in GSI and in oocyte diameters, but ovaries were not fully mature even in mid-March (Figs. 1D, 2). When transferred in early February, spawning was observed under both 18°C 15L and 18°C 10L in three weeks (Fig. 3). In mid-March, avarage GSIs of long and short daylength regimes were 13% and 7%, respectively (Fig. 1E). Both exceeded the average GSI under natural condition (Cochran-Cox test, $p<0.05$).

As for males, average GSIs under natural condition were about 1% from mid-October to early December, but decreased to about 0.7% in early January. Thereafter, GSI increased gradually to 0.9% in mid-March and 1.2% in mid-April. Male GSIs under 18°C 15L or 18°C 10L kept initial value of 1% when transferred in a season from mid-October to late November. Transfer in late December or early February caused increase in GSI to 1.1–1.3% in one month, also irrespective of

		date	Oct. 20	27	Nov. 3	10	17	24	Dec. 1	8	15	22	29	Jan. 5	12	18	25	Feb. 1	8	15	23	Mar. 2
Natural condition	Oct.17	18°C15L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	70	49	30	22	0	23
			(15)					(11)														(8)
		18°C10L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			(15)					(10)														(5)
		NT15L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Natural condition	Oct. 29	18°C15L			0	0	0	0	0	0	0	0	0	+	+	32	21	34	20	20	22	33
					(15)				(10)													(5)
		18°C10L			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
					(15)				(9)													(4)
		18°C NL			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Natural condition	Nov.30	18°C15L							0	0	0	0	0	0	+	105	143	127	123	162	114	141
									(15)					(10)								(6)
		18°C10L							0	0	0	0	0	0	0	0	0	0	0	0	0	0
									(15)					(10)								(5)
Natural condition	Dec.31	18°C15L												0	0	0	0	74	166	121	110	97
														(15)				(9)				(6)
		18°C10L												0	0	0	0	0	0	0	0	0
														(15)				(9)				(6)
Natural temp. 15L	Dec.31	18°C15L												0	0	0	0	139	111	57	46	83
														(15)				(10)				(6)
		18°C10L												0	0	0	0	0	0	0	0	0
														(15)				(10)				(6)
18°C Natural daylength	Dec.31	18°C15L												0	0	0	0	104	78	101	129	122
														(15)				(9)				(5)
		18°C10L												0	0	0	0	0	0	0	0	0
														(15)				(10)				(5)
Natural condition	Feb 3	18°C15L																	0	0	98	166
																			(7)			(7)
		18°C10L																	0	0	36	35
																			(5)			(5)
Natural temp 15L	Feb.3	18°C15L																	0	0	66	155
																			(4)			(4)
		18°C10L																	0	0	0	0
																			(4)			(4)
18°C Natural daylength	Feb.3	18°C15L																	0	+	28	71
																			(15)			(15)
		18°C10L																	0	7	0	0
																			(15)			(15)

FIG. 3. Total number of eggs spawned by *Oryzias latipes* during three days under various temperature photoperiod regimes. Date on the top indicates the middle day. Transfer dates are indicated between regimes. Number of female under each regime is shown in parenthesis. +, spawning was observed; NT15L, natural temperature-15L (15 hr of light/day); 18°C NL, 18°C-natural daylength.

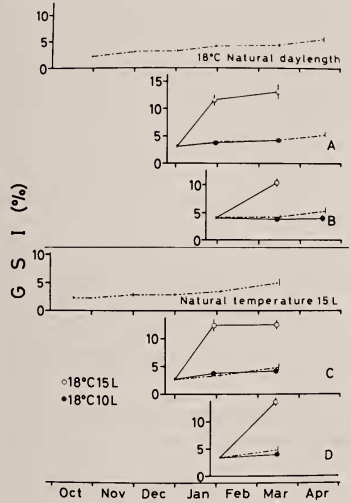
photoperiod.

18°C natural daylength group

Average female GSI slowly increased from 2% in late October to 5% in mid-April (Fig. 4). Until mid-March, oocytes grew as gradually as in the natural condition group (Fig. 5), and no spawning was observed (Fig. 3). In mid-April some fish started spawning (data not shown), but rapid increase in average GSI shown under natural condition did not occur.

Transfer to 18°C 15L in late December brought about rapid ovarian growth. Average GSI reached 11% in one month (Fig. 4A) and spawning began in early February (Fig. 3). Average GSI kept high until mid-March (Fig. 4A). Under 18°C 10L, average GSI increased only to 4% and kept the value till the end of the experiment (Fig. 4A). Oocyte diameters increased a little, but spawning was not observed (Figs. 3, 5). Transfer in early February had almost the same effect as in late December. Under long daylength, fish showed rapid ovarian growth (Fig. 4B) and spawning began in late February (Fig. 3). Under short daylength, average GSI kept low until mid-March (Fig. 4B) and oocyte diameters did not change much (Fig. 5). Minor spawning was observed in mid-February, but ceased in the next week (Fig. 3).

As for males, average GSIs kept high values over 1% under 18°C with natural daylength; 1% in



late October, 1.3% in late November, 1.2% in late December, 1% in early February and 1.2% in mid-April. Transfer to 18°C 15L in late December had no effect on average GSI. Under short daylength, however, GSI slightly decreased to around 1%. The transfer in early February caused GSI increase to 1.4% in mid-March, irrespetive of photoperiod.

Natural temperature 15L group

Average GSI of female fish increased gradually from 2% in October to 5% in mid-March under 15L with natural water temperature (Fig. 4).

Fig. 4. Changes in gonadosomatic index (GSI) of female *Oryzias latipes* reared under 18°C natural daylength or natural temperature 15L, and transferred to 18°C 15L (15 hr of light/day) or to 18°C 10L. The transfer was conducted in late December (A, C) or early February (B, D). Solid lines in graphs A, B, C, D show changes in GSI of the fish transferred from 18°C natural daylength (A, B) or natural temperature 15L (C, D).

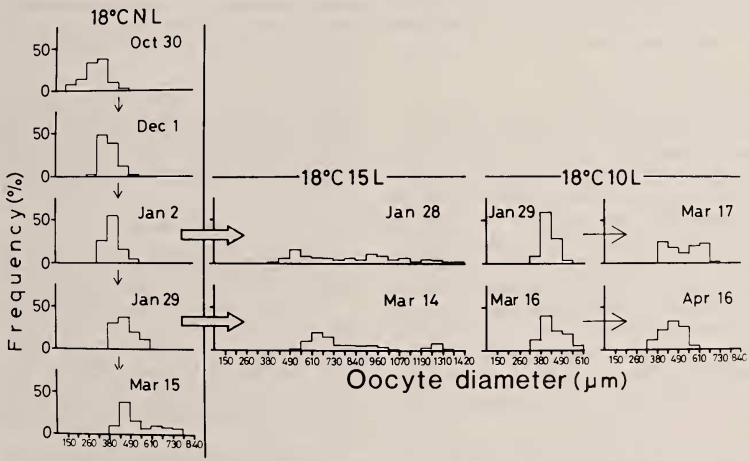


Fig. 5. Changes in distribution of oocyte diameters under 18°C natural daylength (18°CNL) and after the transfer to 18°C 15L or to 18°C 10L. The transfer was conducted in late December and early February, but distributions of oocyte diameters under 18°C NL was examined a few days earlier or later than the transfer. Distributions of oocyte diameters under 18°C 15L or 18°C 10L were examined in one month, and at the end (18°C 10L).

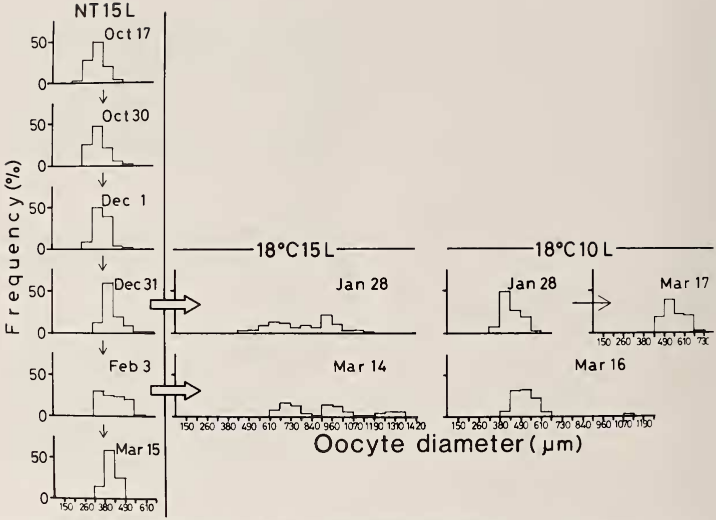


FIG. 6. Changes in distribution of oocyte diameters under natural temperature 15L (NT15L) and after the transfer to 18°C 15L or to 18°C 10L. The transfer was conducted in late December and early February. Distributions of oocyte diameters under 18°C 15L or 18°C 10L were examined in one month, and at the end (18°C 10L).

Oocytes grew slightly in accordance with the increase of GSI (Fig. 6). Spawning did not occur until the end of the experiment (Fig. 3).

When transferred to 18°C 15L in late December, average GSI of female increased rapidly and reached 13% in one month (Fig. 4C). Spawning began in early February (Fig. 3). Under 18°C 10L, however, GSIs kept as low as those of controls (Fig. 4C). Oocyte diameters increased a little by mid-March (Fig. 6), but no spawning was observed (Fig. 3). Transfer to 18°C 15L or to 10L in early February had almost the same effect as in late December (Figs. 4D, 6), except that spawning started under 15L in a shorter period or three weeks after the transfer (Fig. 3).

GSI of males under 15L with natural water temperature decreased slightly from 1% in mid-October to 0.8% in late December, but increased thereafter to 1% in mid-March. When transferred in late December to 18°C 15L or to 10L, male GSI increased, reaching 1.3% and 1.1% respectively. Transfer to 18°C 10L in early February made male GSI decrease to 0.7%. Under 18°C 15L only two males survived, their GSIs being 1.5% and 0.5%.

DISCUSSION

In the natural condition group, ovarian responses of the medaka transferred to 18°C 15L or to 18°C 10L clearly changed with season. Although effects of temperature-photoperiod conditions on the ovarian activity of medaka have already been studied by several workers [9, 13–15], no one has reported seasonal change in ovarian response such as observed in the present study.

Ovarian unresponsiveness in autumn (Fig. 1A) is the same phenomenon as called refractoriness in wild-type medaka [13], *Notropis bifrenatus* [16] and *Heteropneustes fossilis* [17].

After the refractory period, the ovaries came to grow under long daylength. Results of the transfer from the natural condition to 18°C 15L in mid-October (Figs. 1A, 3) suggests that this change in ovarian response requires neither low temperature nor short daylength in winter. Similar results have been reported for wild type medaka [13] and *Notropis bifrenatus* [16].

Marked decline in ovarian photoperiodic response was observed in the natural condition group when transferred in early February. Though females of the 18°C natural daylength group or the natural temperature 15L group in early February had slightly larger oocytes than the natural condition group, they could not mature when transferred to 18°C 10L. Exposure to both low temperature and short daylength in winter seems to be necessary for the maturation independent of photoperiods.

In the natural condition group, the period between transfer to 18°C 15L and beginning of spawning shortened with approach of spring. Egami and Hosokawa [9] observed similar shortening of the period required for maturation in medaka transferred from natural water temperature to 23–26°C, though without photoperiod control, in seasons from autumn to spring. In our experiments, moreover, the females transferred from natural condition to 18°C 15L after late November reached higher GSIs than the females transferred in October, and GSIs of the females transferred from other experimental groups to 18°C 15L after late December were similarly higher than those of the females transferred from natural condition group in October. From these results, short daylength and/or low temperature in winter seem to be profitable for rapid ovarian growth and active spawning under 18°C 15L.

Testicular response to environmental alterations were different from that of ovaries. Seasonal changes in the response were not observed throughout the experiment. GSIs were kept at 1% or more under 18°C 15L or 18°C 10L, and in most cases photoperiods caused no difference in the testicular responses. In this study, however, only GSIs were considered. Histological observation will be necessary for further comments on testicular response.

Concerning environmental regulation of the spawning season of medaka, Awaji and Hanyu [11] showed that rapid gonadal growth and subsequent spawning in spring could be induced by raising water temperature over 14°C, irrespective of photoperiod, in the orange-red type medaka. This coincides with the present results. In the present study, in addition, temperature-photoperiod conditions necessary to induce rapid gonadal growth in medaka were shown to differ with maturational stages of the gonads or environmental career of the employed fish. Necessary period for maturation and average GSI of matured females also differed with these factors.

According to Yoshioka [13, 14, 18, 19], long daylength was necessary for maturation of wild type medaka in every season. Necessity of long daylength was also observed by Chan [15] in the orange-red type medaka. But Egami and Hosokawa [9] could induce ovarian maturation only by raising water temperature. Fish used in these studies were different from one another in its environmental career and the reproductive stage at the beginning of the experiments. On the other hand, Sawara and Egami [20] pointed out possible existence of racial difference in photoperiodic response of gonads in medaka. These circumstances make it difficult to generalize the environmental regulation of gonadal maturation in medaka. But as far as the orange-red type medaka cultured around Tokyo is concerned, environmental regulation of beginning of spawning season can be summarized as follows from the present study. Orange-red type medaka usually begins rapid gonadal growth and subsequent spawning in spring depending on rising water temperature, irrespective of photoperiod. Accidental warm temperature in winter will not induce rapid growth of young oocytes in the absence of long daylength. When ovarian development is delayed by some reasons, long daylength in spring will stimulate ovarian growth and lead to spawning in the regular season.

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